

What is claimed is:

1. A method for detecting at least one target sequence in a sample comprising:

5 combining the sample with a probe set for each target sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the probes in each set are suitable for ligation together when hybridized adjacent to
10 one another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion located between the primer-specific portion and the target-specific portion; and optionally, a ligation agent to form a ligation reaction mixture;

15 subjecting the ligation reaction mixture to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, at least one addressable support-specific portion, and the 3' primer-specific portion;

20 combining the ligation reaction mixture with: (a) at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the 3' primer-specific portion of the ligation product, wherein at least one primer of the primer set further

comprises a reporter group, and (b) a polymerase, to form a first amplification reaction mixture;

subjecting the first amplification reaction mixture to at least one cycle of amplification to generate a first amplification product comprising at least one
5 reporter group;

hybridizing the addressable support-specific portions of the first amplification product to support-bound capture oligonucleotides; and
detecting the reporter group.

10 2. The method of claim 1, wherein the first probe further comprises the addressable support-specific portion.

3. The method of claim 1, wherein the second probe further comprises the addressable support-specific portion.

15 4. The method of claim 1, wherein the probe set further comprises more than one pivotal complement, a pivotal complement that is not the terminal nucleotide of the target-specific portion, or both.

20 5. The method of claim 1, wherein the ligation agent is a ligase.

6. The method of claim 5, wherein the ligation agent is a thermostable ligase.

7. The method of claim 6, wherein the thermostable ligase is *Tth*, *Taq*, or *Pfu* ligase.

5 8. The method of claim 1, wherein each probe set further comprises at least two first probes that differ in the target-specific portion by at least one nucleotide.

10 9. The method of claim 1, wherein each probe set further comprises at least two second probes that differ in the target-specific portion by at least one nucleotide.

15 10. The method of claim 1, wherein the polymerase is a thermostable polymerase.

11. The method of claim 10, wherein the thermostable polymerase is *Taq*, *Pfu*, Vent, Deep Vent, UITma, *Pwo*, or *Tth* polymerase and enzymatically active mutants and variants thereof.

20 12. The method of claim 1, further comprising purifying the ligation product prior to amplification.

13. The method of claim 12, wherein the purifying comprises hybridization-based pullout.

14. The method of claim 12, wherein the purifying comprises gel
5 filtration.

15. The method of claim 12, wherein the purifying comprises dialysis.

16. The method of claim 1, wherein the reporter group comprises a
10 fluorescent moiety.

17. The method of claim 1, wherein the first probe of each probe set further comprises a phosphorothioate group at the 3'-end.

15 18. The method of claim 1, wherein the second probe of each probe set
further comprises a 5' thymidine residue with a leaving group suitable for ligation.

19. The method of claim 18, wherein the 5' thymidine leaving group is tosylate or iodide.

20. The method of claim 1, wherein the first amplification product comprises at least one 5' terminal phosphate; and further comprising:

combining the first amplification product with an exonuclease to form a digestion reaction mixture; and

incubating the digestion reaction mixture under conditions that allow the exonuclease to digest the amplification product to generate single stranded
5 addressable support-specific portions.

21. The method of claim 1, wherein the first amplification reaction mixture comprises at least one first primer or at least one second primer for each primer set, but not both first and second primers of a primer set, and wherein the
10 at least one first primer or the at least one second primer of a primer set, but not both, comprises a reporter group.

22. The method of claim 1, wherein the at least one first probe and the at least one second probe in the probe set further comprise an addressable
15 support-specific portion located between the primer-specific portion and the target-specific portion, and ~~at least~~ two primers of the primer set comprise reporter groups.

23. The method of claim 1, further comprising denaturing the first
20 amplification product to generate single-stranded amplification product.

24. The method of claim 23, wherein denaturing comprises heating the amplification product to a temperature above the melting temperature.

25. The method of claim 24, wherein denaturing comprises chemically denaturing the amplification product.

26. The method of claim 1, wherein the molar concentration of the at least one first primer is different from the molar concentration of the at least one second primer in each primer set.

27. A method for detecting at least one target sequence in a sample comprising:

combining the sample with a probe set for each target sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion; and optionally, a ligation agent to form a ligation reaction mixture;

subjecting the ligation reaction mixture to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the target specific portions, at least one addressable support-specific portion, and the primer-specific portion;

combining the ligation reaction mixture with at least one primer comprising a sequence complementary to the primer-specific portion of the ligation product and a reporter group, and a polymerase, to form an extension reaction mixture;

subjecting the extension reaction mixture to at least one cycle of primer extension to generate a first amplification product comprising at least one reporter group;

hybridizing the addressable support-specific portions of the first amplification product to support-bound capture oligonucleotides; and detecting the reporter group.

28. The method of claim 27, wherein the first probe further comprises the addressable support-specific portion.

29. The method of claim 27, wherein the second probe further comprises the addressable support-specific portion.

30. The method of claim/27, wherein the ligation agent is a ligase.

31. The method of claim 30, wherein the ligation agent is a thermostable ligase.

32. The method of claim 31, wherein the thermostable ligase is *Tth* or *Taq* ligase.

33. The method of claim 27, wherein each probe set further comprises at least two first probes that differ in the target-specific portion by at least one nucleotide.

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34. The method of claim 27, wherein each probe set further comprises at least two second probes that differ in the target-specific portion by at least one nucleotide.

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35. The method of claim 27, wherein the polymerase is a thermostable polymerase.

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36. The method of claim 35, wherein the thermostable polymerase is *Taq*, *Pfu*, or *Tth* polymerase.

37. The method of claim 27, further comprising purifying the ligation product prior to amplification.

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38. The method of claim 37, wherein the purifying comprises hybridization-based pullout.

39. The method of claim 37, wherein the purifying comprises gel filtration.

40. The method of claim 37, wherein the purifying comprises dialysis.

41. The method of claim 27, wherein the reporter group comprises a
5 fluorescent moiety.

42. The method of claim 27, wherein the first probe of each probe set
further comprises a phosphorothioate group at the 3'-end.

10 43. The method of claim 27, wherein the second probe of each probe
set further comprises a 5' thymidine residue with a leaving group suitable for
ligation.

15 44. The method of claim 43, wherein the 5' thymidine leaving group is
tosylate or iodide.

45. A method for detecting at least one target sequence in a sample
comprising:

20 combining the sample with a probe set for each target sequence, the
probe set comprising (a) at least one first probe, comprising a target-specific
portion and a 5' primer-specific portion, and (b) at least one second probe,
comprising a target-specific portion and a 3' primer-specific portion, wherein the
probes in each set are suitable for ligation together when hybridized adjacent to

subjecting the second amplification reaction mixture to at least one cycle of amplification to generate a second amplification product comprising at least one reporter group;

5 hybridizing the addressable support-specific portions of the second amplification product to support-bound capture oligonucleotides; and detecting the reporter group.

46. The method of claim 45, wherein the at least one first probe and the at least one second probe in the probe set further comprise an addressable support-specific portion located between the primer-specific portion and the target-specific portion, and at least two primers of the second amplification reaction mixture primer set comprise reporter groups.

47. A probe suitable for ligation comprising: a 5'-end, a 3' end, a target-specific portion, a primer-specific portion, and an addressable support-specific portion located between the primer-specific portion and the target-specific portion.

48. The probe of claim 47, further comprising a free phosphate group at the 5'-end.

49. The probe of claim 47, further comprising a phosphorothioate group at the 3'-end.

50. The probe of claim 47, further comprising a thymidine residue at the 5'-end with a leaving group suitable for ligation.

5 51. The probe of claim 50, wherein the 5' thymidine leaving group is tosylate or iodide.

52. A kit for detecting at least one target sequence in a sample comprising:

10 at least one probe set for each target sequence to be detected, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the probes in each probe set are suitable for ligation together when hybridized adjacent to one
15 another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion located between the primer-specific portion and the target-specific portion; and optionally,
a ligation agent.

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53. A kit according to claim 52, further comprising a set of nucleotide primers, the primer set comprising (i) at least one primer comprising the sequence of the 5' primer-specific portion of the probe, and (ii) at least one

primer comprising a sequence complementary to the 3' primer-specific portion of the probe, wherein at least one primer of the primer set further comprises a reporter group; and a polymerase.

5 54. A kit according to claim 52, further comprising a support, the support comprising capture oligonucleotides capable of hybridizing with addressable support-specific portions of the probes or with sequences complementary to the addressable support-specific portions of the probes.

10 55. A kit according to claim 54, wherein the polymerase is a thermostable polymerase.

 56. A kit according to claim 55, wherein the thermostable polymerase is *Taq*, *Pfu*, *Vent*, *Deep Vent*, *UITma*, *Pwo*, or *Tth* polymerase.

15 57. A kit according to claim 52, wherein the ligation agent is a ligase.

 58. A kit according to claim 57, wherein the ligase is a thermostable ligase.

20 59. A kit according to claim 58, wherein the thermostable ligase is *Tth* or *Taq* ligase.

60. A kit for detecting at least one target sequence in a sample comprising:

at least one probe set for each target sequence to be detected, each probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion located between the primer-specific portion and the target-specific portion.

61. A kit according to claim 60, further comprising a support, the support comprising capture oligonucleotides capable of hybridizing with addressable support-specific portion of the at least one probe or with sequences complementary to the addressable support-specific portions of the at least one probe.

62. A kit according to claim 60, further comprising a primer set, the primer set comprising (i) at least one primer comprising the sequence of the 5' primer-specific portion of the first probe, and (ii) at least one primer complementary to the 3' primer-specific portion of the second probe, and wherein

at least one primer of the primer set further comprises a reporter group; and a polymerase.

63. A kit according to claim 60, wherein the reporter group comprises a
5 fluorescent moiety.

64. A kit according to claim 60, wherein the first probe of each probe set further comprises a phosphorothioate group at the 3'-end.

10 65. A kit according to claim 60, wherein the second probe of each probe set further comprises a 5' thymidine residue with a leaving group suitable for ligation.

15 66. A kit according to ~~claim~~ claim 65, wherein the 5' thymidine leaving group is tosylate or iodide.

20 67. A kit according to claim 60, wherein the first probe of each probe set further comprises a phosphorothioate group at the 3'-end and wherein the second probe of each probe set further comprises a 5' thymidine residue with a leaving group suitable for ligation.

68. A kit according to ~~claim~~ claim 67, wherein the 5' thymidine leaving group is tosylate or iodide.

addressable support-specific portions of the probes or with sequences complementary to the addressable support-specific portions of the probes.

73. A kit according to claim 71, further comprising at least one primer
5 complementary to the primer-specific portion of the second probe and a reporter group; and a polymerase.

74. A kit according to claim 71, wherein the reporter group comprises a fluorescent moiety.

75. A kit according to claim 71, wherein the polymerase is a thermostable polymerase.

76. A kit according to claim 75, wherein the thermostable polymerase
15 is *Taq*, *Pfu*, Vent, Deep Vent, UITma, *Pwo*, or *Tth* polymerase or enzymatically active mutants or variants thereof.

77. A kit according to claim 71, wherein the ligation agent is a ligase.

78. A kit according to claim 77, wherein the ligase is a thermostable
20 ligase.

79. A kit according to claim 78, wherein the thermostable ligase is *Tth* or *Taq* ligase.

80. A kit according to claim 71, wherein the first probe of each probe set further comprises a phosphorothioate group at the 3'-end.

81. A kit according to claim 71, wherein the second probe of each probe set further comprises a 5' thymidine residue with a leaving group suitable for ligation.

82. A kit according to claim 81, wherein the 5' thymidine leaving group is tosylate or iodide.

83. A kit according to claim 71, wherein the first probe of each probe set further comprises a phosphorothioate group at the 3'-end and wherein the second probe of each probe set further comprises a 5' thymidine residue with a leaving group suitable for ligation.

84. A kit according to claim 83, wherein the 5' thymidine leaving group is tosylate or iodide.

85. A kit according to claim 71, wherein each probe set further comprises at least two first probes that differ in the target specific portion by at least one nucleotide.

5 86. A kit according to claim 71, wherein each probe set further comprises at least two second probes that differ in the target specific portion by at least one nucleotide.

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10 87. A method for detecting at least one target sequence in a sample comprising:
combining the sample with a probe set for each target sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the
15 probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion located between the primer-specific portion and the target-specific portion; and optionally, a ligation agent to form a ligation reaction mixture;
20 subjecting the ligation reaction mixture to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-

specific portions, at least one addressable support-specific portion, and the 3' primer-specific portion;

combining the ligation reaction mixture with: (a) at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the 3' primer-specific portion of the ligation product, wherein at least one primer of the primer set further comprises a reporter group, and (b) a polymerase, to form a first amplification reaction mixture;

subjecting the first amplification reaction mixture to at least one cycle of amplification to generate a first amplification product comprising at least one reporter group;

separating the components of the first amplification product; and
detecting the reporter group.

88. The method of claim 87, wherein separating comprises electrophoresis, gel filtration, mass spectroscopy, or HPLC.

89. The method of claim 87, wherein the first probe further comprises the addressable support-specific portion.

90. The method of claim 87, wherein the second probe further comprises the addressable support-specific portion.

91. The method of claim 87, wherein the ligation agent is a ligase.

92. The method of claim 91, wherein the ligation agent is a
5 thermostable ligase.

93. The method of claim 92, wherein the thermostable ligase is *Tth*, *Taq*, or *Pfu* ligase.

94. The method of claim 87, wherein each probe set further comprises at least two first probes that differ in the target-specific portion by at least one nucleotide.

95. The method of claim 87, wherein each probe set further comprises at least two second probes that differ in the target-specific portion by at least one nucleotide.

96. The method of claim 87, wherein the polymerase is a thermostable polymerase.

97. The method of claim 96, wherein the thermostable polymerase is *Taq*, *Pfu*, *Vent*, *Deep Vent*, *UITma*, *Pwo*, or *Tth* polymerase and enzymatically active mutants and variants thereof.

98. The method of claim 87, further comprising purifying the ligation product prior to amplification.

5 99. The method of claim 98, wherein the purifying comprises hybridization-based pullout.

100. The method of claim 87, wherein the reporter group comprises a fluorescent moiety.

101. A method for detecting at least one target sequence in a sample comprising:

combining the sample with a probe set for each target sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence, and wherein at least one probe in each probe set further comprises an addressable support-specific portion; and optionally, a ligation agent to form a ligation reaction mixture;

subjecting the ligation reaction mixture to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another

113. The method of claim 112, wherein the purifying comprises hybridization-based pullout.

5 114. The method of claim 101, wherein the reporter group comprises a fluorescent moiety.

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